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PATENT
Docket No.: 19603/1426 (CRF D-2238A)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Goldman et al.
Serial No.	:	09/282,239
Cnfrm. No.	:	To Be Assigned
Filed	:	March 31, 1999
For	:	A METHOD FOR ISOLATING AND PURIFYING OLIGODENDROCYTES AND OLIGODENDROCYTE PROGENITOR CELLS

Examiner:
Richard Hutson

Art Unit:
1652

DECLARATION OF STEVEN A. GOLDMAN UNDER 37 C.F.R. §1.132

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

I, STEVEN A. GOLDMAN, pursuant to 37 C.F.R. § 1.132, declare:

1. I received B.A. degrees in Biology and Psychology from the University of Pennsylvania in 1978, a Ph.D. degree in Neurobiology from Rockefeller University in 1983, and an M.D. degree from Cornell University Medical College in 1984.

2. I am a Professor of Neurology and Neuroscience at Weill Medical College at Cornell University and an Attending Neurologist at New York Presbyterian Hospital.

3. I am a named inventor of the above patent application.

Kirschenbaum Article

4. I am a co-author of Kirschenbaum, et. al., "*In Vitro* Neuronal Production and Differentiation by Precursor Cells Derived from the Adult Human Forebrain," Cerebral Cortex 6: 576-89 (1994) ("Kirschenbaum").

5. The study described in Kirschenbaum was carried out in my laboratory and I was the senior, supervising scientist on the study; therefore, I fully understand this work. Kirschenbaum cultures samples of adult temporal lobes under conditions suitable for neuronal differentiation, while exposed to ^{3}H-thymidine. These samples were incubated for 7-28 days, stained for neuronal and glial antigens, and autoradiographed. Neuron-like cells were found in explant outgrowths and monolayer dissociates of the subependymal zone and periventricular white matter but not the cortex. A small number of Map-2^{+} and Map-5^{+}/glial fibrillary acidic protein^{-} cells did incorporate ^{3}H-thymidine, suggesting neuronal production from precursor mitosis. However, the O4^{+} oligodendrocytes were postmitotic. Even though the abstract of Kirschenbaum states that "O4^{+} oligodendrocytes, although the predominant cell type, were *largely* postmitotic (emphasis in original)", I said this only because I am generally reluctant to make conclusions in absolute terms. Nevertheless, it is clear from the following statement on page 582 of Kirschenbaum that, in fact, *all* of the oligodendrocytes were post-mitotic:

> These O4^{+}/GFAP$^{\pm}$ cells were mitotically quiescent; among a sample of 8044 such cells, culled from four plates of subcortical white matter (2011 ± 858.6 O4^{+} cells/plate, mean ± SD), *none* incorporated ^{3}H-thymidine *in vitro*, despite the frequent observation of ^{3}H-thymidine-labeled astrocytes in the same plates (emphasis in original).

The failure of the Kirschenbaum study to identify mitotic oligodendrocyte progenitor cells caused me to continue working to identify and produce such cells. These efforts were ultimately successful in producing the invention of the present application.

Bottenstein Patent

6. U.S. Patent No. 5,276,145 to Bottenstein ("Bottenstein") is directed to substantially purified preparations containing a neural progenitor regulatory factor that is important in regulating and coordinating production of oligodendrocytes and type 2 astrocytes. The identification of this factor was carried out with brain cells derived from neonatal rats of 1-3 days of age. These cells represented a mixture of cell types, that included "progenitors", "Type 2 Astrocytes", "Early Oligodendrocytes", "Late Oligodendrocytes", "Total Oligodendrocytes", "Type 1 Astrocytes", and "Microglia".

7. There are fundamental differences between the biology of rat and human oligodendrocyte progenitor cells. These are unaddressed in Bottenstein, which discusses findings

limited to neonatal rat brain. Whereas rat oligodendrocytes appear to retain mitotic potential, human oligodendrocytes do not (see Kirschenbaum). As a result, the oligodendrocyte progenitor cell of the rat brain cannot be considered homologous to its human counterpart. In particular, methods that permit the selective extraction and/or growth of oligodendrocyte progenitors from the rat brain do not differentiate between oligodendrocyte progenitor cells and mature oligodendrocytes able to re-enter the mitotic cycle. In humans, these constitute two discrete phenotypes, lineally related but temporally distinct. Our present invention teaches the selective acquisition of a highly enriched – to virtual purity - mitotically-competent oligodendrocyte progenitor cell pool, operationally separate and distinct from post-mitotic or mature oligodendrocytes.

8. Bottenstein was directed at the enrichment of glial progenitor cells from newborn rat brain. Newborns have an abundant population of still-developing oligodendrocyte progenitor cells that may constitute a significant fraction of all of the cells in neonatal brain tissue. Bottenstein reported that >30% of the cells of its tissue dissociates expressed the marker of this phenotype. With the addition of B104 conditioned media and the neural progenitor regulatory factor, this fraction increased to just over 40%. The nature of these cells is that of a still-mixed pool, in that the following populations appear to be represented by Bottenstein's data: astrocytes, oligodendrocytes, and a mixture of oligodendrodendroglial lineage cells of widely different developmental stages.

9. In contrast to the cells acquired from newborn rats using the Bottenstein protocol, the present invention is achieved with a procedure that permits, in both young and old humans, the selective extraction of progenitor cells strongly biased to oligodendrocytic phenotype, and allows the purification of these cells, including those from tissues in which they are scarce (e.g., postnatal and adult brain tissues harboring <1% of the desired oligodendrocyte progenitor cell type). In Example 5 of the present patent application, we reported the virtual purification of oligodendrocyte progenitor cells from tissues with a P/CNP2 promoter-targeted FACS-defined incidence of <1%. This constituted a far greater enrichment of the oligodendrocyte progenitor cell (i.e. 170-fold) than that achieved by Bottenstein (i.e. less than 1.5-fold) and yields a far more pure product of oligodendrocyte progenitor cells.

10. In contrast to Bottenstein, the human oligodendrocyte progenitor cell populations achieved through our protocols are virtually pure as to phenotype. Compare Figure 5B to its control, Figure 5A. In Figure 5A, the gated single cell represents the false-positive sort incidence. Such incidences constitute <1% of the frequency of events noted in Figure 5B, indicating >99% purity of the P/CNP2:hGFP-sorted oligodendrocyte progenitor cells. This can be modulated as a function of sort speed to achieve any desired degree of purity, the trade-off being lower yields as higher degrees

of purification are achieved. By virtue of the high-purity extraction attainable by fluorescence-activated cell sorting, the progenitor cells we produce are never exposed to paracrine factors released by other cells, after removal from tissue. This permits their maintenance in an undifferentiated and phenotypically-unbiased state, in contrast to the mixed cellular milieu afforded by Bottenstein, in which non-oligodendrocytic and non-glial progenitor-derived phenotypes remain abundant.

11. As a result of these considerations, the selective propagation of mitotically-active oligodendrocyte progenitor cells from the neonatal rat brain, as taught by Bottenstein, does not predict the successful isolation of mitotic oligodendrocyte progenitor cells from postnatal or adult human brain tissue.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/31/02

Steven A. Goldman, M.D., Ph.D.

EXPRESS MAIL CERTIFICATE

DOCKET NO. : **19603/4230 (CRF D-2238B)**

APPLICANTS : **Steven A. Goldman and Su Wang**

TITLE : **A METHOD FOR ISOLATING AND PURIFYING OLIGODENDROCYTES AND OLIGODENDROCYTE PROGENITOR CELLS**

Certificate is attached to the **Declaration of Steven A. Goldman Under 37 C.F.R. §1.132** of the above-named application.

"EXPRESS MAIL" NUMBER: **EI989354488US**
DATE OF DEPOSIT: **June 10, 2002**

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, Virginia 22202 **Box CPA Application**.

Shawn A. Lockett
(Typed or printed name of person mailing paper or fee)


(Signature of person mailing paper or fee)